Meniscus Tissue Engineering on the Nanoscale

From Basic Principles to Clinical Application

Brendon M. Baker, BS
Albert O. Gee, MD
Neil P. Sheth, MD
G. Russell Huffman, MD, MPH
Brian J. Sennett, MD
Thomas P. Schaer, VMD
Robert L. Mauck, PhD

ABSTRACT: The meniscus is a fibrocartilaginous tissue uniquely adapted to enable load transmission in the knee. Although the meniscus was once considered a useless remnant of joint formation, removal of all or part of the meniscus initiates osteoarthritis. Surgical repair methods focus on fragment stabilization or biologic enhancement of healing. An alternative approach based on tissue-engineering principles involves the development of new materials for implantation. Our meniscus tissue-engineering efforts aim to recapitulate the architectural features and mechanical anisotropies essential to native tissue function. We use a novel scaffold production technology called *electrospinning*, in which organized three-dimensional arrays of ultrafine biodegradable fibers are generated. Using these scaffolds as micropatterns for directed growth, we have generated constructs with mechanical properties and architectural features comparable to native meniscus. This review details our progress and outlines the remaining hurdles that must be addressed to translate this work into clinical implementation.

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Mr Baker and Drs Gee, Sheth, Huffman, Sennett, and Mauck are from the McKay Orthopaedic Research Laboratory, Department of Orthopaedic Surgery, University of Pennsylvania, Philadelphia, and Dr Schaer is from the Comparative Orthopaedic Research Laboratory, Department of Clinical Studies, New Bolton Center, University of Pennsylvania, School of Veterinary Medicine, Kennett Square, Pa. Mr Baker and Dr Mauck are also from the Department of Bioengineering, University of Pennsylvania, Philadelphia, Pa.

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Correspondence: Robert L. Mauck, PhD, Assistant Professor of Orthopaedic Surgery and Bioengineering, McKay Orthopaedic Research Laboratory, Department of Orthopaedic Surgery, University of Pennsylvania, 36th Street and Hamilton Walk, Philadelphia, PA 19104.

STRUCTURE AND FUNCTION OF THE KNEE MENISCUS

Anatomy, Extracellular Matrix, and Cell Biology of the Knee Meniscus

The knee meniscus is a wedge-shaped fibrocartilaginous structure that transmits loads, absorbs shock, and enhances joint stability. These semilunar tissues reside between the femur and the tibia within the medial and lateral compartments of the knee (Figure 1). Formerly considered fibrous remnants of joint formation, these unique tissues have come to be appreciated for their critical role in the mechanical function of the knee joint; their importance is demonstrated by their presence in numerous animal species, including mammals, birds, and amphibians.[6]

The meniscus is described as fibrocartilaginous because it shares characteristics of both fibrous tissues (ie, tendon and ligament) and cartilaginous tissues (ie, articular cartilage). In bulk, the meniscus extracellular matrix contains 85% to 95% dry weight collagen, of which >90% is type I,[35] with the remainder consisting mostly of types II, III, V, and VI.[60] Proteoglycans make up <2% to 3% of the dry weight, 8-fold less than that in articular cartilage.[1,36,60] The meniscus, like articular cartilage, is highly hydrated, with 72% to 77% of the wet weight composed of water.[1] In addition, the tissue ranges from being heavily vascularized in the outer periphery to completely lacking

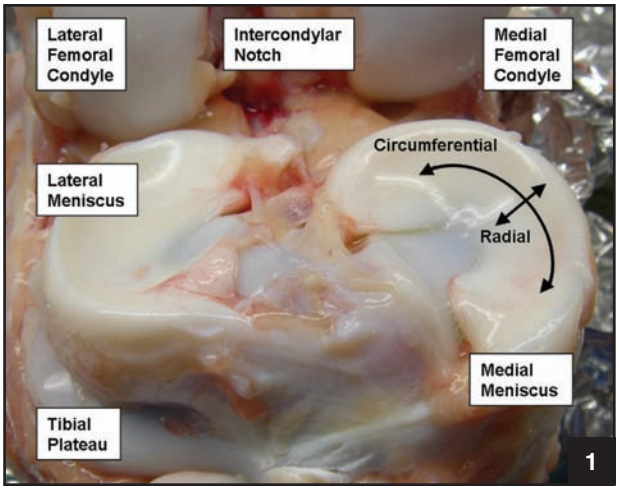

Figure 1. Bovine femoro-tibial joint demonstrating the relative position and shape of the medial and lateral menisci, with respect to the femoral condyles and the underlying tibial plateau. Radial and circumferential orientations of the collagen fibrous architecture are indicated by arrows.

in blood supply in the inner region.[5] In the adult meniscus, the inner avascular region is more hyaline like, whereas the outer vascular region is more fibrous in appearance. The resident cells of the tissue are known collectively as *meniscal fibrochondrocytes*. These cells sparsely populate the tissue substance in the adult and function to maintain and remodel the extracellular matrix (Figure 2A).[1,60] Meniscal fibrochondrocytes are an inhomogeneous population, with cells derived from the different meniscal regions exhibiting distinct morphologies. For example, meniscal fibrochondrocytes in the peripheral region have numerous cellular processes, whereas those in the inner avascular region have a rounded morphology.[43]

Mechanical Properties of the Meniscus

Given its central position in the knee, there has been significant interest in elucidating the mechanical properties of the meniscus. Paramount to its mechanical function, the unique architecture of the meniscus consists of circumferentially oriented collagen fibers interspersed with radial collagen "tie" fibers (Figures 2B, 2D, and 2E).[36,68] Proteogylcans are present at low levels in general, and are highest in the inner avascular zone.[21] As may be expected for such a fiber-reinforced matrix, the mechanical properties of this tissue are highly anisotropic (ie, different in opposing directions) and strongly dependent on the prevailing fiber direction.[20,79] This can be seen in the tensile stress-strain response of samples oriented in the circumferential direction, compared with those samples oriented in the radial direction. Circumferential samples show a pronounced "toe" region common to fiber-reinforced tissues and a higher linear modulus thereafter (Figures 2C and 2F). Radial samples are relatively linear in their stress-strain response, with a much lower modulus. The tensile properties of the meniscus range from 48 to 259 MPa in the circumferential direction and 3 to 70 MPa in the radial direction, depending on anatomic location and species.[20,36,71,79] The compressive properties of the meniscus are low, relative to those of articular cartilage (ie, 100 to 400 kPa, approximately half).[83] The meniscus, although less stiff in compression, is also much less permeable than articular cartilage,[36] suggesting that the tissue is optimized to enhance congruency, load distribution, and shock absorption across the joint.[79]

Physiologic Loading of the Meniscus

The main functions of the meniscus are to transmit and distribute the compressive load between the femur and the tibial plateau, increase joint congruency, stabilize the joint, and improve articular cartilage nutrition and lubrication.[39] These functions are achieved by the unique load transfer that occurs between the more hyaline inner region and the more fibrous outer region of the meniscus. When the joint is loaded vertically, axial loads from the femoral condyles impinging on the wedge-shaped portion of the meniscus are redirected laterally. Lateral extrusion of the meniscus is resisted by the osseous anchorage of the anterior and posterior horns,[79] generating hoop stresses within the dense network of circumferentially oriented collagen fibers. With normal use, the menisci transmit 50% to 100% of the loads in the knee (ie, multiples of body weight),[3] with tensile deformations limited to 2% to 6%.[75,87]

MENISCUS DAMAGE AND DEGENERATION

Because the meniscus continually operates in a rigorous mechanical environment, damage is common with most patients over the age of 45 having some evidence of meniscal scarring. The annual incidence of meniscal injuries requiring surgical intervention is estimated to be 60 to 70 per 100,000 per year, with most occurring in male patients between ages 21 to 30 and in female patients between ages 11 and 20.[41,64] The cause of meniscal tears in young people is usually trauma, whereas in people over the age of 40, tears more often result from degeneration.[40] Meniscal damage can manifest in a variety of forms, including circumferential and horizontal tears (ie, in which fracture occurs between collagen bundles) or radial tears (ie, in which collagen bundles are disrupted). Different tears arise from different origins: trauma leads to bucket handle circumferential tears, whereas degeneration often results in horizontal and radial tears.

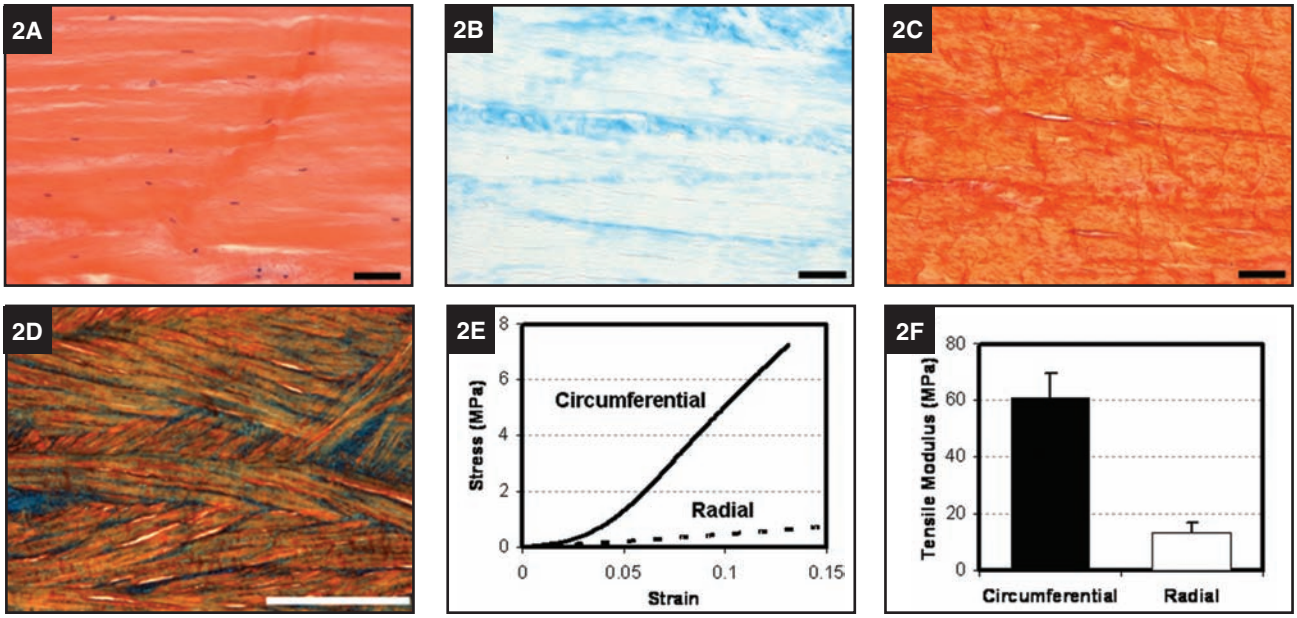

Figure 2. Hematoxylin-eosin staining (original magnification ×20) showing limited cellularity (purple nuclei) and abundant extracellular matrix (orange matrix) in circumferential samples obtained from the central body of the meniscus (A). Alcian blue staining for proteoglycans is limited to the interstitial areas outside of dense collagen bundles (B). Dense collagenous matrix of the meniscus is visualized through picrosirius red staining (C). Polarized light microscopy analysis of picrosirius red-stained sections reveals a highly organized network of collagen fibers within the macroscopic circumferential bundles (D). Typical stress-strain response of meniscus samples tested in the circumferential and radial directions (E). The tensile modulus in the circumferential direction is approximately 6 times higher than in the radial direction (F).

As with other dense connective tissues, the menisci are optimized for mechanical functionality at the expense of healing capacity. After birth and during joint loading, the vasculature of the meniscus recedes to the periphery, cellularity decreases, and the collagenous extracellular matrix grows denser (Figure 3).[24] As vascularity is associated with healing responses, juvenile meniscus injuries heal readily, whereas in the adult, complete healing only is observed in the vascularized periphery.[69] More complicated tears, such as those that interrupt the circumferential fibers in the avascular zone, have a poor long-term prognosis.

One clinical strategy for accelerating healing in avascular regions is the drilling of channels to promote blood vessel invasion.[7] Even when healing does occur, radial tears often result in fibrovascular scars containing disorganized collagen with inferior mechanical properties.[63] Restoration of fiber arrangement has never been documented, and the injured segment of these tears is commonly resected.[29] Mechanical compromise of the meniscus due to damage or resection decreases the contact area and increases stress concentrations on the adjacent articular surfaces.[2,72] Altered loading can in turn lead to detriment to the articular cartilage, inspiring osteoarthritic changes, such as osteophyte formation and joint space narrowing.[69,72]

CURRENT CLINICAL PRACTICE IN MENISCUS REPAIR

Surgical Methods for Meniscus Repair

Many surgical techniques have been adopted for augmenting the natural healing process of the meniscus. In some procedures, rasping of the synovium or the intercondylar notch is performed to increase the vascular response.[37,64] It has been suggested that if repair of the avascular zone is attempted, a healing enhancement technique should be considered.[29] The most common and successful repair technique is suturing and stabilizing the meniscal tears, bringing the torn portions back into apposition using either open repair or arthroscopic surgery.[90] These suturing techniques include inside-out (ie, inside the joint capsule to the meniscus periphery) or outside-in (ie, from the periphery to the internal space) approaches.[29,76] There has also been a growing interest in devices that use an all-inside arthroscopic technique for meniscal repair. These include commercially available biodegradable fixation devices, including screws, arrows, and darts, that provide for shorter surgical times, easier approaches for implantation, and reduced surgical risk.

Meniscus Allografts

Another approach to meniscus repair is allograft transplantation of a whole fresh-frozen or cryopreserved me-

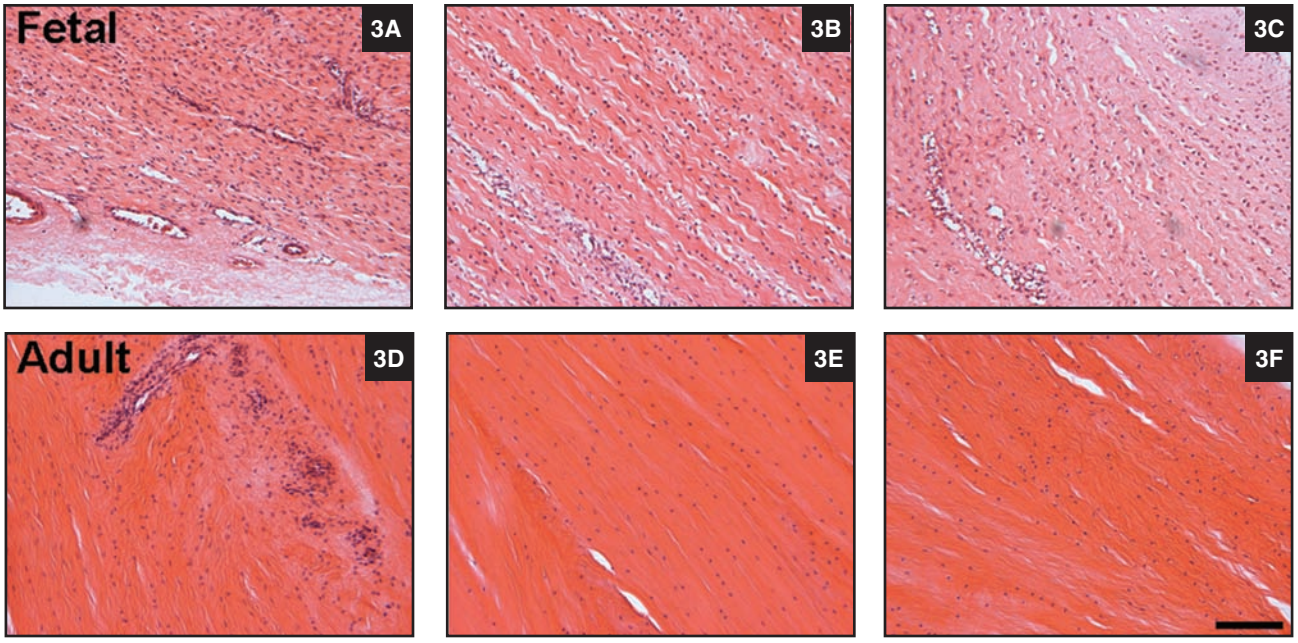

Figure 3. Histology of the meniscus with increasing age. Hematoxylin-eosin staining of adult and fetal meniscus samples showing region-specific (outer zone [A, D]; middle zone [B, E]; inner zone [C, F]) and age-dependent variations in matrix content, cellularity, and blood vessel distribution. With increasing age, the menisci become less cellular (ie, fewer nuclei) and less vascular (ie, fewer vessels observed), particularly in the inner region (original magnification ×10).

niscus from a human donor. These tissue transplants, derived from cadaveric sources, are fixed to the tibial plateau with bone blocks or tunnels. Whole meniscus transplants can restore some aspects of load transmission after total meniscectomy. However, several studies have reported cartilage degeneration following implantation, most likely attributable to improper sizing of the implants.[34,8,49,61] A recent study showed that the contact area of and resulting load transmission through cartilage and meniscus varied considerably from normal if allografts were >10% bigger or smaller than the original tissue size.[31] In addition, nonanatomic positioning of meniscus transplants can significantly affect load transmission,[92] and may explain some of these variations in clinical findings. Allografts also raise concerns regarding the methods of cryopreservation, graft sterility, and the proper method of fixation.[49] Clinical results of meniscal allografts suggest that function is only partially restored and that poor integration makes the grafts susceptible to premature failure.[73] These findings highlight the need for a functional repair strategy other than total replacement for the clinical treatment of meniscal tears.

Enhancing Endogenous Repair

In addition to surgical fixation and replacement techniques, some emerging therapies have been designed to enhance endogenous repair. One of the first methods emerged from the observation that some repair occurred in canine meniscus where a fibrin clot had adhered to the cut surface.[8] This observation led to the introduction of vascular channels to improve access, as well as to the implantation of fibrin clots.[9] In these first studies, a defect was created in the avascular region of the meniscus and was filled with a fibrin clot. These clots and the factors within served as both chemotactic and mitogenetic agents, encouraging cellular in-growth and division within the wound site.[5] With clot treatment, healed tissue remained markedly different from native tissue, although it was highly cellular and of a better quality than that found in untreated lesions.[5] In a similar study, devitalized meniscal chips with autologous chondrocytes[66] were implanted in avascular longitudinal tears in a porcine model.[67] This approach resulted in improved tissue formation compared with sutured controls, although complete healing of defects was not observed. However, these findings suggest a role for cell-based methods for enhancing meniscus repair.

TISSUE ENGINEERING OF THE KNEE MENISCUS

Tissue Engineering Strategies

Despite advances in surgical technique and fixation devices, a pressing clinical demand exists for new strategies for repairing the knee meniscus. As of 2002, close to 1000 meniscus transplants were being performed each

year in the United States,[46] with an even larger number of transplantations possible if this tissue were more readily available. To fill this need, a number of tissue engineering approaches have emerged, combining materials, cells, or drug and growth factor delivery systems to enhance the repair and replacement of damaged meniscus. For example, in approaches that mirror allograft procedures, whole acellular polymeric menisci have been fabricated,[51.52,81] as have meniscus-shaped constructs formed from dense cell pellets shaped around molds.[10] Recently, sophisticated magnetic resonance imaging and injection molding techniques have been coupled to create anatomic constructs composed of cell-seeded alginate hydrogels.[17] Other cell-seeded scaffolds, such as poly-L-lactic acid (PLLA) porous foams with embedded carbon fibers,[91] polyglycolic acid felts,[47] and macroporous polycaprolactone-polyurethane and polycaprolactone-PLLA constructs have also been investigated. Such structures can be produced with compressive properties similar to native tissue while promoting cellular ingrowth after implantation,[42] although these implants often result in cartilage erosion in in vivo studies.[89] Natural biopolymers have also been used, including type I collagen glycosaminoglycan-supplemented scaffolds,[82] perichondral tissue,[19] and porcine small intestinal submucosa grafts.[26] Of note, one biologic scaffold, the collagen meniscus implant, which is produced from decellularized bovine Achilles tendon, was recently used in a randomized clinical trial in humans. These implants showed promise in maintaining knee function in patients with degenerative meniscus damage,[77] although they were less effective in the treatment of traumatic lesions. While many acellular and cell-based tissue-engineering therapies have emerged, no single approach has shown functional in vivo repair and the reconstitution of fiber architecture of the native tissue.

Nanofiber-Based Meniscus Tissue Engineering

We have focused our meniscus tissue engineering efforts on aligned nanofibrous scaffolds produced by electrospinning. This novel fabrication methodology is becoming increasingly prevalent in tissue engineering applications.[22,57,70] In this process, charge repulsion in a polymer droplet overcomes surface tension, resulting in the emission of a jet in the presence of a strong electric field.[74] This jet is drawn through the high-voltage gradient toward a grounded collecting plate, forming ultra-thin fibers (50 to 1000 nm) similar in diameter to the native extracellular matrix.[30] With this small-length scale, a single cell can interact with many fibers simultaneously, mimicking its normal interactions with its pericellular extracellular matrix environment (Figure 4A). This small fiber diameter has been shown to promote matrix-forming activities in seeded cells; chondrocytes on PLLA nanofibers produce more matrix than when seeded on PLLA microfibers.[54] With increasing production time, randomly oriented fibers accumulate to form meshes with pore sizes of 2 to 465 μm[55] and void volumes of 80% to 90%.[56] Production of meshes by electrospinning has been conducted with numerous synthetic and natural polymers.[18] Mechanically, these nonaligned scaffolds exhibit isotropic mechanical properties that are reflective of the mechanical properties of their polymer composition. For example, crystalline polymers such as poly-lactide-coglycolide produce stiff meshes, whereas scaffolds composed of polycaprolactone are 10-fold less stiff but remain elastic over a wider range.[53] With the increasing number of polymers available for electrospinning, a wide range of mechanical and degradation characteristics can be achieved in these novel scaffolds.

Fabrication of Anisotropic Nanofibrous Scaffolds

To engineer dense connective tissues such as the meniscus, additional methods are required to replicate the native tissue architecture and mechanical anisotropy. Nanofibrous scaffolds can serve this role when collection and fabrication strategies are modified to promote fiber alignment. A common method for instilling alignment in these scaffolds is by replacing the static grounded collecting plate with a rotating mandrel (Figure 4B).[85] We and others have used the method to organize fibers within forming nanofibrous meshes, and to precisely control their architecture.[11,28,56] In one recent study,[56] we produced poly(ϵ-caprolactone) scaffolds that varied in the degree of fiber alignment by electrospinning onto a target rotating with a surface velocity ranging from 0 to 9.3 m/s (Figures 4C through 4E). We measured the fiber angles relative to the spinning direction in scanning electron micrographs and found that the increased speed of the target resulted in increasingly parallel fibers. Near-complete alignment was achieved when rotation speeds reached 9.3 m/s. When these acellular scaffolds were mechanically tested in tension, the fiber alignment had a profound effect on the mechanical properties of scaffolds. A 27-fold increase in modulus in the fiber direction, compared with that perpendicular to the fiber direction, was observed when fibers were deposited at 8 m/s. Thus, by controlling the speed of the target onto which nanofibers are electrospun, one can dictate the starting mechanical anisotropy and tune the mechanical properties to mimic native tissue features. Recently, we used similar methods to produce scaffolds with the prevailing fiber axis oriented at various angles off of the long axis of the scaffold, a key feature for application in annulus fibrosus tissue engineering.[62] In addition, we have developed new spinning methods to create multipolymer blended composites[13] and photocrosslinkable nanofibers from a diverse biodegradable polymer li-

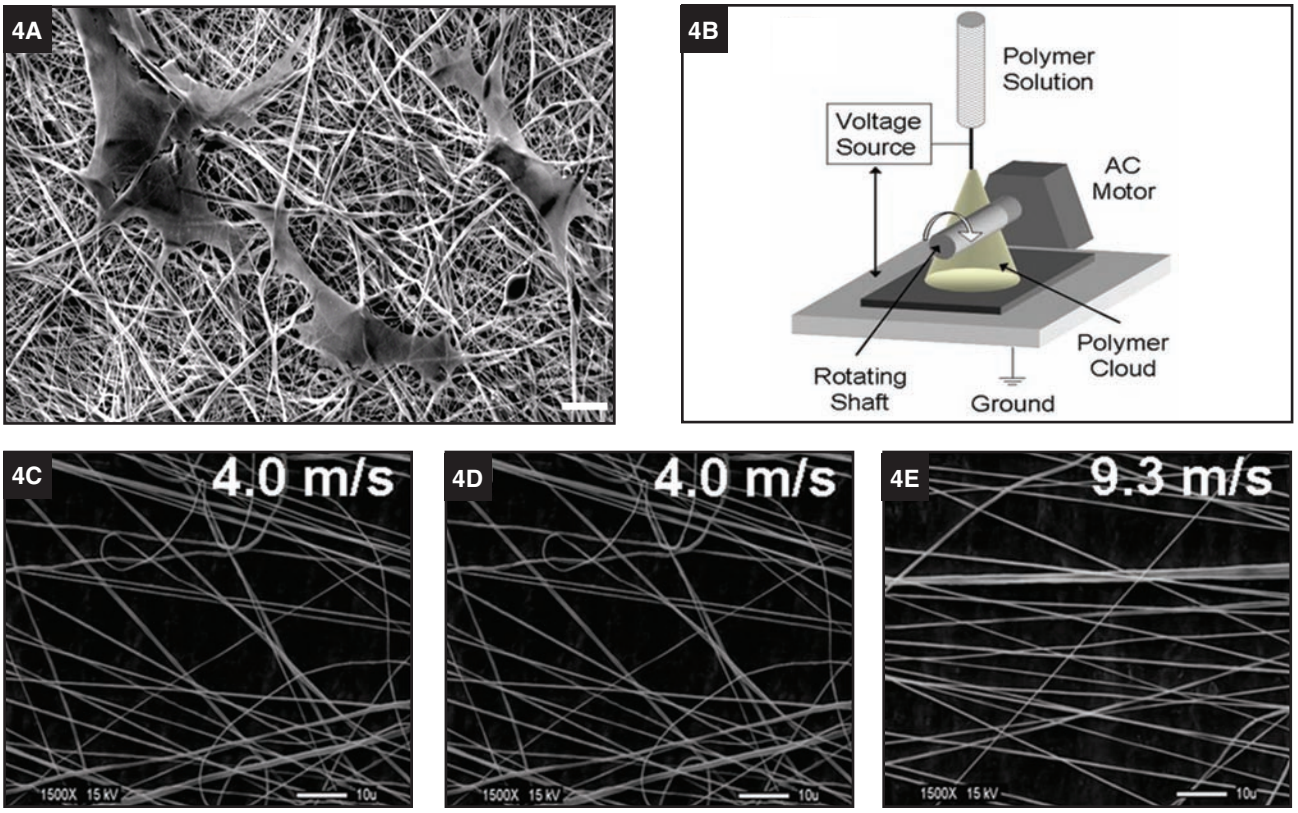

Figure 4. Electrospun scaffolds for meniscus tissue engineering. Scanning electron micrograph of bovine mesenchymal stem cells on a nonaligned nanofibrous scaffold composed of polycaprolactone is shown (A) (original magnification ×2000). Schematic showing an electrospinning device incorporating a rotating collecting mandrel to generate aligned fibrous meshes (B). Mandrel velocity (top right) dictates the degree of alignment in forming nanofibrous scaffolds (fibers collected at increasing mandrel velocities [C-E]) (original magnification ×1500).

brary,[84] further expanding the application of this novel scaffolding technology.

Cell-Mediated Maturation of Nanofibrous Meniscus Constructs

Although starting mechanical properties of scaffolds are important, the ideal engineered construct will eventually be replaced in its entirety by newly deposited extracellular matrix produced by cells implanted along with the scaffold, or by cells that invade the scaffold in situ. As mentioned above, a number of cell types have been evaluated for their potential to attach to and colonize nanofibrous scaffolds of varying composition. This nanofibrous topography can influence cell behavior and dictate cell morphology.[14] In a recent study[14] focused on meniscus tissue engineering, we assessed the long-term maturation of nanofibrous scaffolds seeded with bovine meniscus fibrochondrocytes or with multipotential bovine mesenchymal stem cells derived from bone marrow. Cell-mediated matrix deposition and construct mechanical properties were evaluated on both nonaligned and aligned scaffolds over 10 weeks of culture in defined medium.[59] At the outset of the study, aligned scaffolds encouraged the seeded cells to preferentially align in the predominant fiber direction, whereas cells on nonaligned scaffolds were randomly oriented. In addition, aligned scaffolds initially had a higher tensile modulus (approximately 12 MPa) than did their nonaligned counterparts (approximately 4 MPa). If seeded with mesenchymal stem cells or meniscal fibrochondrocytes, both aligned and nonaligned scaffolds increased in tensile properties over time (Figure 5). Of note, aligned constructs increased by approximately 10 MPa whereas nonaligned constructs increased by only approximately 1 MPa, regardless of cell type. With time in culture, cells deposited increasing amounts of fibrocartilaginous extracellular matrix (ie, proteoglycan and collagen). Histology of cross-sections of these constructs indicated that cells infiltrated into the outer two-thirds of the scaffold and matrix deposition increased with time. The most interesting finding in this study was that although cells on nonaligned and aligned scaffolds produce comparable amounts of matrix, marked increases in tensile properties were observed only on aligned scaffolds. Polarized light microscopy of sections taken of en face sections (in the scaffold plane)

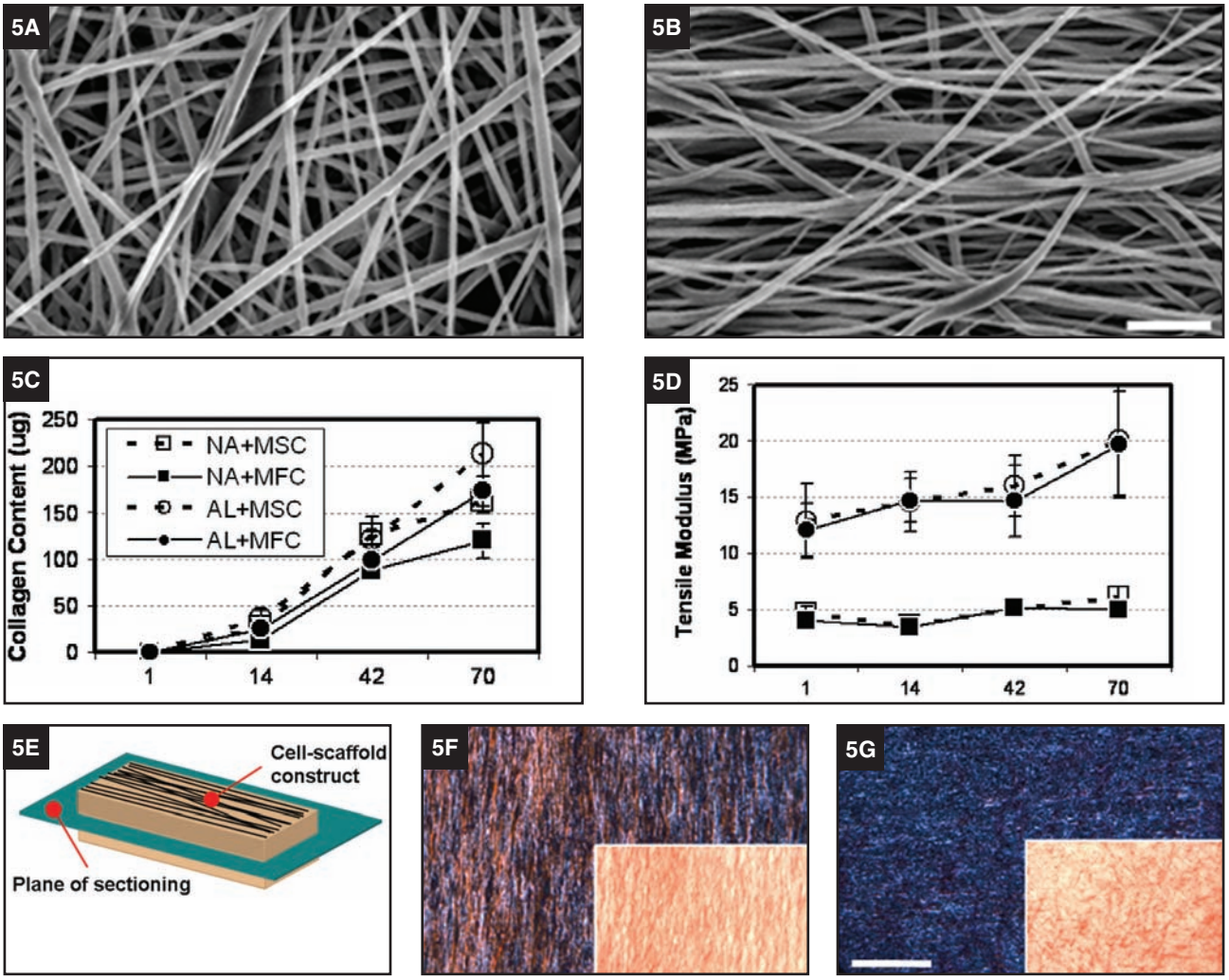

Figure 5. Nanofiber alignment promotes functional extracellular matrix accumulation in engineered meniscus constructs. Nonaligned (NA) (A) and aligned (AL) (B) nanofibrous scaffolds were seeded with bovine meniscal fibrochondrocytes (MFC) or mesenchymal stem cells (MSC) (original magnification ×2000). Collagen deposition over time was independent of the scaffold type (C). Tensile properties of aligned cell-seeded constructs show larger increases with time in culture than nonaligned constructs similarly seeded and cultured (D). Under polarized light, en face sections (E) of aligned constructs (F) (original magnification ×10) revealed alignment of newly deposited collagen (orange hue). Collagen alignment was absent in nonaligned constructs (G) (original magnification ×10). Picrosirius red staining showed similar overall collagen deposition in both nonaligned and aligned constructs (insets).

showed that the instructive pattern of aligned scaffolds led to the deposition of aligned collagen. The production of an organized extracellular matrix resulted in a greater enhancement of tensile properties in aligned constructs, compared with nonaligned constructs.

Meniscus Tissue Engineering with Human Meniscus Cells

To further the clinical application of these scaffolds, we recently evaluated human meniscus cells derived from surgical debris for their potential to generate engineered constructs for implantation.[15] For this study, human meniscal fibrochondrocytes were isolated from 10 donors ranging in age from 18 to 84 who presented with a variety of meniscus lesions. These spanned traumatic injuries in the younger patient, degenerative fissures in middle-aged patients, and menisci removed from osteoarthritic patients undergoing total joint replacement. After sufficient expansion in monolayer, these human meniscal fibrochondrocytes colonized scaffolds in a similar fashion, as did the juvenile bovine meniscal fibrochondrocytes and mesenchymal stem cells in our previous studies. Mechanical properties increased significantly for cells derived from each donor during a

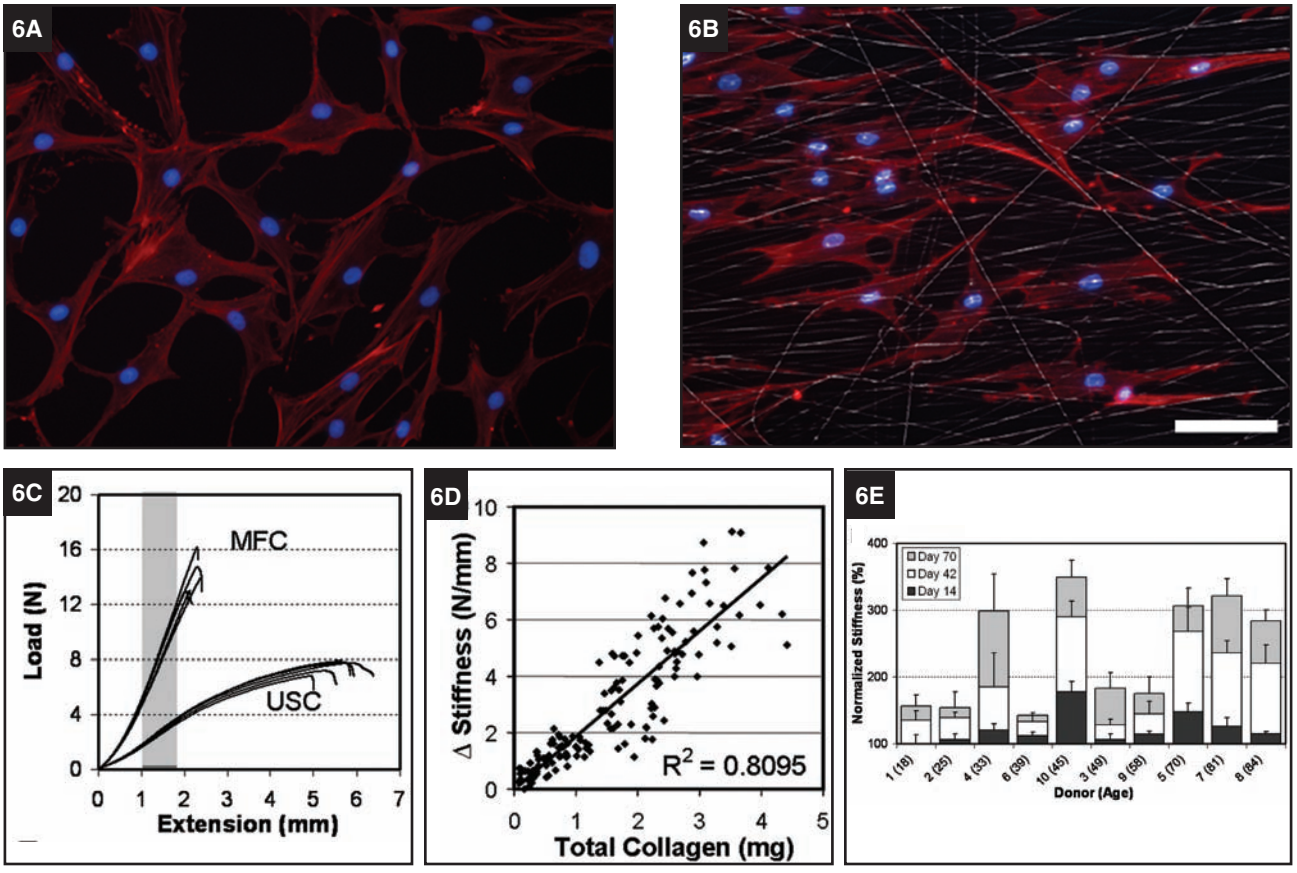

Figure 6. Human meniscal fibrochondrocytes (MFC) deposit functional matrix on aligned nanofibrous scaffolds. Culture-expanded meniscal fibrochondrocytes (A) derived from 10 donors took on an elongated morphology when seeded onto aligned scaffolds (B) (original magnification 20×). During 10 weeks of culture, seeded constructs developed a nonlinear stress-strain response greater than unseeded controls (USC) (C). This increase in properties corresponded well with newly deposited collagen content (D). Although donor-to-donor variability was noted, all donors significantly increased in mechanical properties over the culture period (E).

10-week period (Figure 6). For the best performing donors, construct properties reached 40 MPa, a level close to that of the native tissue. In addition, these constructs developed a nonlinear stress-strain response reminiscent of the native tissue. Increases in construct mechanical properties correlated strongly with the amount of collagen generated by the cells. However, as in previous studies, the long culture time required for cell colonization (42-70 days) and the need for enhanced void space for more robust matrix accumulation motivated further modifications to our scaffold production methods. We have recently reported the inclusion of sacrificial fibers composed of a water soluble polymer (polyethylene oxide) within the slow-degrading poly(epsilon-caprolactone) network.[13] With hydration, a defined fraction of sacrificial fibers is removed from the composite scaffold, increasing the pore size to promote cell infiltration while maintaining a high degree of anisotropy. These tunable, multipolymer constructs containing sacrificial elements can improve cell colonization while preserving nanoscale cell-scaffold interactions, preinstilled structural anisotropy, and mechanical properties within the maturing scaffold. Tuning these composites may further expedite the rate of maturation of constructs in vitro and after in vivo implantation.

Integration and Anatomic Form

Although the results presented above indicate the potential of fiber-aligned fibrous constructs to guide neotissue growth, clinical application will require the fabrication of constructs with anatomic relevance. The meniscus fills a unique anatomic space, ensuring congruency between the rounded femoral condyle and the flat tibial plateau. Therefore, tissue engineering strategies must recapitulate the microscale and nanoscale topography of the tissue, as well as the macroscale anatomic form, to promote functional regeneration. In addition, these scaffolds must have the capacity to integrate with native tissue when a subtotal implant is fabricated.

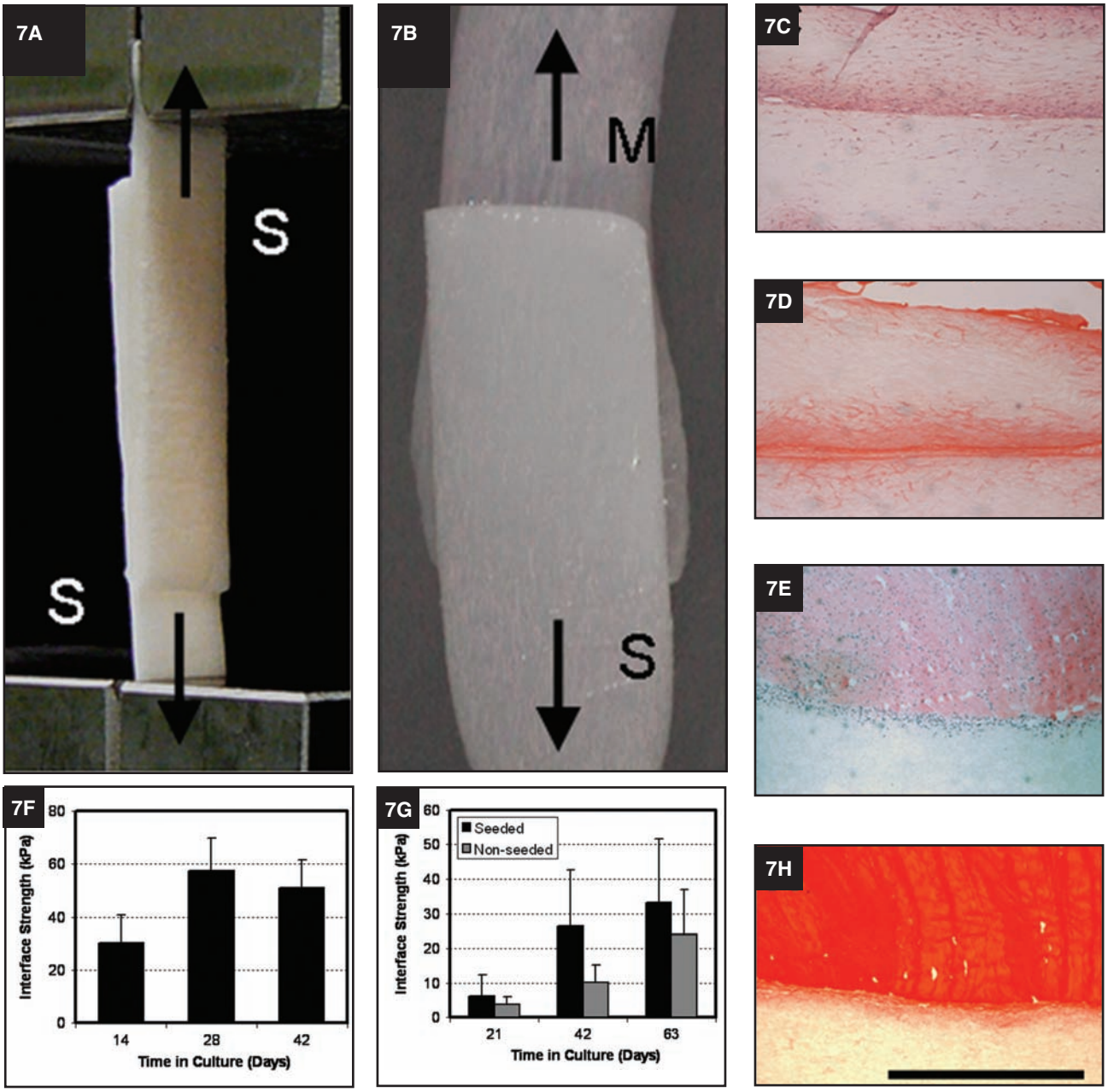

Figure 7. Scaffold-to-scaffold (A) and scaffold-to-meniscus (B) constructs were formed and cultured for ⩽9 weeks in vitro. In both cases, mechanical strength of the interface increased with time (F, G), and new matrix was deposited at the interface (Scaffold-to-scaffold: C, D; Meniscus-to-scaffold: E, H) (hematoxylin-eosin [C,E] and picosirius red staining [D,H,] original magnification ×10). (Abbreviations: S, scaffold; M, meniscus.)

To address the first issue, we examined cell-mediated mechanical integration of meniscal fibrochondrocyte-seeded and mesenchymal stem cell-seeded constructs when held in apposition with one another.[16] In this formulation, each layer serves as a focal source of cells to hasten full construct colonization, and is a platform for the construction of larger, multilamellar structures. To address the second issue, we also evaluated integration strength between nanofibrous constructs and native meniscus tissue.[80] Integration strength between the layers was assessed using a lap test, with maximum force normalized to the overlap area. Results of these studies demonstrate that after 2 weeks of combined culture (scaffold-to-scaffold or scaffold-to-meniscus), a stable union formed. Additional incubation times up to 9 and 24 weeks led to continuing increases in interface strength, reaching several hundred

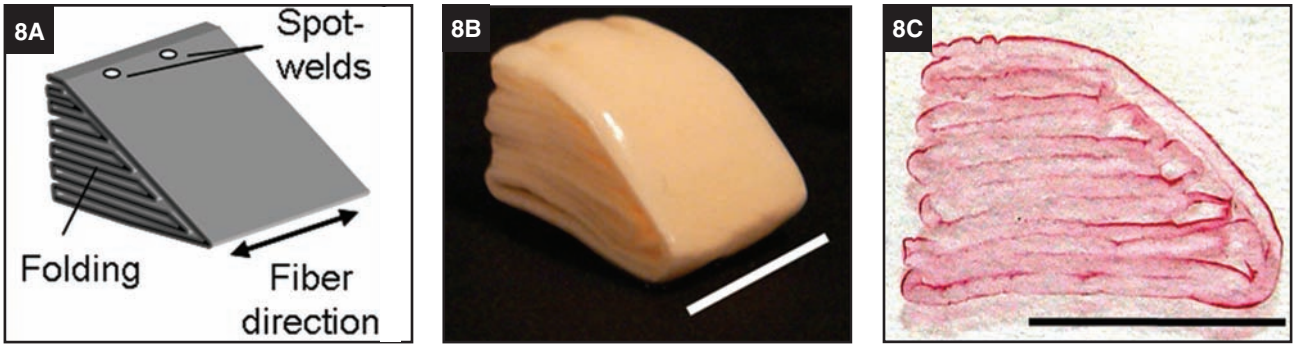

Figure 8. Construction algorithms were developed to generate constructs with three-dimensional anatomic form (wedge shape) while preserving microscopic fibrous architecture (A). Mesenchymal stem cell-seeded wedge-shaped constructs took on a meniscus-like appearance after 3 weeks in culture (B), with collagen deposition occurring between each layer (C) (scale bar = 1 cm).

kilopascals for scaffold-to-scaffold interfaces (Figure 7).[16] These values are consistent with recent reports of meniscus-to-meniscus integration strengths measured with long-term in vitro culture of circular meniscus biopsies that are immediately replaced in annular structures.[45] Histological analysis of these forming interfaces showed a steady increase in extracellular matrix deposition over time. Extending these studies to achieve anatomic relevance, we also fabricated wedge shapes using a custom folding technique. This approach preserved fiber circumferential orientation while approximating anatomic form. To stabilize constructs initially, a spot-welding approach was adopted in which a heated probe (80°C) fused the adjacent acellular layers.[12] Nanofiber-based anatomic wedges were formed and seeded with 10 million mesenchymal stem cells via syringe. After 3 weeks in culture, wedges were harvested and stained for cell localization and matrix deposition. As shown in Figure 8, the wedge construct takes on a tissue-like appearance with matrix deposition occurring at each layer. These results are similar to those observed in bilamellar constructs at this early time point. Collectively, these results show that nanofibrous constructs can be formed into structures with anatomic relevance while anisotropic mechanical and architectural features are preserved. These data also show that with time a stable mechanical interface will form between adjacent layers and scaffold and native tissue via cell-mediated matrix deposition, offering potential for direct meniscus repair applications.

ANIMAL MODELS OF MENISCUS REPAIR

As new enabling technologies are developed to restore the normal load-bearing role of the knee meniscus, appropriate animal models are required to test engineered construct efficacy in the context of the demanding load-bearing environment of native tissue. In addition, because the primary role of the meniscus is to protect the underlying cartilage, engineered constructs must be tested in a model that is sufficiently aggressive to demonstrate cartilage preservation. Several animal species have been used to study meniscus structure, function, and mechanobiology. For example, in rabbits, meniscal fibrochondrocyte biosynthetic activities are altered with joint instability.[43,44] In rats, exercise changes the meniscus biochemical content.[88] Although useful, these animal models are generally too small to assess changes in the mechanical properties of the meniscus and adjacent tissues or to evaluate meniscus repair devices. Thus, most meniscus repair studies use canine, caprine, or ovine models. Compared with canine models, ovine models are particularly useful for evaluating meniscus injury and repair, as their menisci are closer in size to that of human beings and show similar loading patterns.[48] Consistent with smaller mammals, proteoglycan production is decreased in canine meniscus with immobilization,[32] and cartilage erodes with meniscus damage or removal in dogs and sheep.[33,93]

Several recent studies aimed at meniscus repair and replacement have used these animal models. Studies by Kobayashi et al[52] using a polyvinyl alcohol hydrogel in a rabbit total meniscectomy model and Kelly et al[50] using a commercial hydrogel meniscal implant (Salumedica, Atlanta, Ga) in a sheep total meniscectomy model both found lower Mankin scores at 2 years and 4 months, respectively, when compared with controls. Recent studies have also shown efficacy with long-term implantation of biologic small intestinal submucosa patches and porous polymeric foams in canine and sheep models.[25,26,86] Cook et al[25,26] examined the use of small intestinal submucosa for partial meniscal defects in a dog model and found that the meniscus-like tissue that was regenerated achieved 55% of the compressive modulus of the contralateral control meniscus tissue at 1 year, offering some protection to the underlying cartilage, although degenerative changes were observed relative to

controls. Tienen et al[86] showed that meniscus replacements composed of porous biodegradable Estane polymers (BF Goodrich Chemical, NV Westerlo-Oevel, Belgium) increased in compressive modulus at 6 months in a total meniscectomy model in dogs. Unfortunately, similar to small intestinal submucosa, the scaffolds were unable to achieve the mechanical properties of normal meniscus tissue at the final time point. In most ovine studies, empty defects show deleterious changes in cartilage within 6 weeks of injury, whereas meniscus and Achilles tendon grafts,[61] collagen-based meniscus implants,[58] polymer foams,[23] and acellular hydrogels[50] have shown some degree of cartilage protection after 3 to 52 weeks.

OUTCOME ASSESSMENT FOR MENISCUS TISSUE ENGINEERING

The animal studies described above show promise for the evaluation of new meniscus repair devices for both partial defect repair and whole meniscus implantation. Standard measures of meniscus volume and cartilage health, including India ink staining of surfaces, histological analysis of both the implant and the integration site, and mechanical assessment of neomeniscus and cartilage tissue, provide valuable information regarding the efficacy of the engineered repair. In addition, clinical methods of analysis (lameness scores) provide information about recovery of function after implantation. However, a drawback to these studies is the length of time required to demonstrate real efficacy. In human beings, although removal of increasing amounts of meniscus tissue during meniscectomy procedures hastens the rate of cartilage erosion and joint space narrowing, these processes still take years to fully develop into symptomatic osteoarthritis. Most animal studies, although thorough and rigorous, do not generally test for these lengthy times due to cost considerations. These long time intervals also lengthen the crucial engineering process of design and revision.

To expedite this engineering process, new methods should be adopted. For example, a recent study by Cottrell et al[27] used a sheep model of partial and complete meniscectomy to map pressure distribution with meniscus removal. In that study, sheep knee joints were mounted in a knee testing device reprogrammed to apply normal sheep gait dynamics and forces across the joint while simultaneously recording the location and magnitude of load transmission with an electronic pressure sensor. Such techniques can readily be converted to assay the load transmission in engineered constructs immediately upon implantation, as well as in joints after various periods of in vivo integration and maturation.

Correlating long-term clinical, mechanical, and histological outcomes from studies such as these with changing design parameters in the original implant may decrease the revision time in technology development and expedite the development and refinement of repair methods. When technologies transition to human application, as has recently occurred for the collagen meniscus implant,[77] thorough evaluation is limited to patient surveys of function, second-look arthroscopies, and biopsies of neotissue, as well as magnetic resonance imaging of joint structures. Incorporating these same analysis tools into animal models may help to unite these model systems, resulting in improved preclinical model systems, ultimately enhancing the predictability of clinical outcome during translational research.

FUTURE DIRECTIONS AND CHALLENGES

The knee meniscus is a complex tissue with structural properties that allow it to perform its physiologic role over a lifetime of use in a demanding mechanical environment. When damaged, intrinsic meniscus repair processes are limited, particularly in the poorly vascularized regions of the tissue. These damaged regions are commonly resected—a surgical process that increases the probability of subsequent cartilage erosion in the affected compartment. The limitations of current clinical treatment methods has generated a growing interest in the generation of a functional tissue-engineered meniscus construct. Numerous cell-based and biomaterial-based processes have been developed for enhancing meniscus repair. Natural and synthetic scaffolds that recreate the mechanical properties and architecture of native tissue have shown promise in creating replacement constructs in vitro and in vivo.

We present a novel fabrication method that has potential to direct the formation of organized extracellular matrix that better recapitulates the native tissue. An additional hurdle is the identification of the most appropriate cell source and the mobilization of endogenous cells. We have demonstrated the potential of meniscus fibrochondrocytes from native tissue, as well as the ability of mesenchymal stem cells, to elaborate a fibrocartilaginous matrix. Clinical application will require evaluation of the most appropriate cell source, as well as the most efficient means of their isolation and application. It remains to be definitively established whether a cell-based strategy is required for success, and several divergent strategies all hold promise. For example, acellular scaffolds could be implanted and colonized in situ by endogenous cells. Alternatively, previously isolated bone marrow-derived stem cells could be incorporated into scaffolds and matured constructs implanted at the time of resection and repair. Finally, meniscal fibrochondrocytes could be isolated during meniscectomy and used in cultivating a functional meniscus construct to be implanted in a second repair sur-

gery. Preclinical trials using large animal models will be required to establish the most appropriate path for repair. Continued optimization of these tissue-engineering efforts may also include the use of in vitro bioreactors and anabolic culture environments to produce constructs that more completely recapitulate the form and function of the native tissue. Additional practical considerations such as scale-up and quality control of fabricated constructs, new surgical approaches to enable arthroscopic replacement, and the modularity of fabricated constructs must be investigated. In addition, methods for the fixation of these constructs into the repair site, as well as the proper rehabilitation regimen that allows for continued in vivo maturation and integration, will also be required. Although still in its early stages, the realization of a functional engineered meniscus construct based on nanofibrous scaffolds may enhance the repair of avascular defects in the meniscus, correcting what is otherwise a progressively debilitating and untreatable orthopedic condition.

POTENTIAL CLINICAL APPLICATION

Dense connective tissues of the musculoskeletal system are constructed to optimize mechanical functionality; however, they do so at the expense of the healing capacity. Meniscus injuries are extremely prevalent, and meniscus repair and removal procedures constitute the most frequent orthopedic operation each year in the United States.[40] Currently, >250,000 total knee replacements are performed in the United States each year, and meniscus injury is a key mediator of the osteoarthritic progression that results in cartilage erosion.[4] As of 2002, close to 1000 full meniscus transplants were being performed each year in the United States, with an even larger number of transplantations possible if tissue were more readily available.[46]

The promise of meniscus tissue engineering has been demonstrated by the recent positive finding using collagen-based implants in human clinical trials.[77,78] Our data reveal the capacity of nanofibrous scaffolds to direct new tissue formation that matches native tissue architecture and to foster maturation processes that recapitulate some aspects of native tissue mechanics, and points to their potential in engineering meniscus replacements. Ongoing work to improve cell infiltration rates will expedite the regenerative capacity of these constructs after implantation. In addition, these scaffolds may be coupled with a controlled biofactor release to tailor in vivo matrix deposition and vascular invasion. By fabricating scaffolds in the shape of the meniscus, this technology will be readily transitioned to preclinical trials in validated large animal models of efficacy and, eventually, clinical implementation. Successful completion of this work will markedly improve the prospects of meniscus regeneration and integration and address what is an otherwise untreatable pathology directly linked to the progression of joint disease in the knee.

Although the focus of the work described in this article is the knee meniscus, these same enabling technologies may be incorporated into strategies for the repair of other fiber-reinforced tissues, such as tendons, ligaments, and intervertebral discs. These tissues all share a common developmental process, wherein fiber alignment is established early in development, and adult function relies on increasing fiber reinforcement through guided extracellular matrix deposition. Thus overcoming the existing limitations in meniscus tissue engineering with these novel scaffolds will significantly affect the development of engineered replacements for other similar tissues. Together, these enabling technologies have great potential to dramatically enhance the future of allograft science and tissue engineering via the provision of new fiber-reinforced constructs for implantation.

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